(c-type) inactivated state, is coupled to the opening and closing of the activation (intracellular) gate. Opening the activation gate by applying an external stimulus results in a transient current before the selectivity filter undergoes a spontaneous transition toward the non-conductive inactivated conformation that blocks the passage of ionic current through the channel. Only after closing the activation gate by removing the external stimulus will the selectivity filter return back to its original conductive conformation, resetting the filter so that it may once again pass current. While it is thought that recovery from the non-conductive inactivated state involves subtle conformational changes of the selectivity filter, the reason why this process can take up to several seconds, which is extremely slow on the molecular timescale, is not understood. Our results from a series of MD simulations reveal the selectivity filter is sterically locked in the inactive conformation for more than 15 microseconds by 12 buried water molecules, 3 for each subunit, that are strongly bound behind the filter. Even the presence of a few of these buried waters appears to lock the selectivity filter in the inactive conformation, blocking the filter from returning to a conductive conformation until the buried waters spontaneously vacate each subunit. Such an event would be rare, stretching the process of recovery to the timescale of seconds. To validate this mechanism, experiments were conducted where an osmotic stress was applied on the extracellular side of the channel to decrease the probability of waters occupying the cavities located behind the filter. As predicted, this accelerated the rate of recovery from slow inactivation.

Platform: Membrane Receptors & Signal Transduction I

134-Plat

Differences in the Allosteric Interaction between Agonists and GMP-PNP in Monomers and Oligomers of the M2 Muscarinic Cholinergic Receptor Fused to GαI1

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The nature of the signaling complex between G protein-coupled receptors (GPCRs) and G proteins remains unclear. GPCRs can exist as oligomers, yet monomers can activate G proteins when reconstituted in nanodiscs; the interaction with the G protein often is considered transient, yet some evidence suggests that the RG complex remains intact during signaling. Two classes of sites typically are observed in the binding of agonists to GPCRs in natural membranes, and guanylyl nucleotides such as GMP-PNP effect an apparent interconversion from higher (K_H) to lower affinity (K_L) without affecting affinity *per se*. We have examined three models of a stable RG complex in which the M_2 muscarinic receptor is fused to $G\alpha_{i1}$ via different linking sequences. Each fusion protein was expressed in *Sf*9 cells and characterized as an oligomer in digitonin-solubilized preparations, as a purified monomer in solution, and as an oligomer reconstituted in phospholipid vesicles. In the oligomeric state, the agonist oxotremorine-M recognized two classes of sites; GMP-PNP progressively increased the low-affinity fraction without affecting K_H or K_L. In the monomeric state, oxotremorine-M similarly recognized two classes of sites, most of which were of high affinity (F_H=71%); GMP-PNP progressively increased K_H, as expected for an allosteric interaction between two sites, without affecting K_L or F_H. The effect of GMP-PNP in monomers required the presence of DTT; the nucleotide-independent sites of low affinity may represent a subpopulation in which communication between the receptor- and α_{i1}-domains has been compromised. Only the oligomeric form of the fusion protein mimics the behavior of GPCRs in natural membranes, suggesting that signaling *in vivo* proceeds via a stable complex comprising multiple equivalents of receptor and G protein. (Supported by HSFO and CIHR)

135-Plat

Functional Comparison of Monomers and Tetramers of the M_2 Muscarinic Receptor

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M_2 muscarinic receptors were purified as monomers from *Sf*9 cells and reconstituted as monomers in nanodiscs and as tetramers in phospholipid vesicles. In the absence of G proteins, monomers in solution and in nanodiscs appeared homogeneous ($n_H \approx 1$), as revealed by the inhibitory effect of agonists and antagonists on binding of the antagonist *N*-[^{3}H]methylscopolamine. Reconstituted tetramers appeared homogenous to antagonists ($n_H > 0.80$) and heterogeneous to agonists ($n_H < 0.80$). The heterogeneity was modelled as two classes of sites ($\log\ldots IC_{50(High)}$, $\log\ldots IC_{50(Low)}$) and quantified as the product of the difference in affinity ($\Delta\log IC_{50}$) and the fraction of sites exhibiting higher affinity (F_H) (*i.e.*, $F_H\Delta\log\ldots IC_{50}$). The resulting values correlated with the corresponding values of $F_H\Delta\log\ldots IC_{50}$ and the intrinsic activities reported previously for binding and response, respectively, in natural membranes ($p < 0.0001$). In the presence of G proteins, reconstituted monomers and tetramers exhibited sensitivity to the guanyl nucleotide GMP-PNP. With tetramers, increasing concentrations of GMP-PNP caused an upward shift in the binding profile of the agonist oxotremorine-M, an effect that emerged from the model as an interconversion of labeled sites from high to low affinity with little or no change in affinity *per se*. With monomers, increasing concentrations of GMP-PNP caused a lateral rightward shift that emerged as an increase in the value of $\log\ldots IC_{50}$. The vertical shift displayed by tetramers mirrors the patterns observed previously in native membranes, while the lateral shift displayed by monomers seems to be artefactual. This observation, taken together with the correlation among agonists between efficacy and the heterogeneity of reconstituted tetramers devoid of G protein, suggests that oligomers—most likely tetramers—are the functional unit of the M_2 muscarinic receptor in nature. (Supported by HSFO and CIHR)

136-Plat

Enhanced Conformational Sampling of M_2 Muscarinic Acetylcholine Receptor for Designing Selective Allosteric Drugs

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Muscarinic acetylcholine receptors are members of the superfamily of G-protein coupled receptors (GPCRs) and play critical roles in both the central and parasympathetic nervous systems. They are important drug targets for the treatment of a spectrum of diseases including abnormal heart rate, asthma, chronic obstructive pulmonary disease, Alzheimer's disease, Parkinson's disease and schizophrenia. Enhanced conformational sampling of M2 muscarinic receptor is achieved via accelerated molecular dynamics (aMD) simulations. The simulation output structures are clustered into representative conformers that are structurally distinct from each other. Potential allosteric binding sites are then identified by mapping the surface of the receptor conformers. This enables virtual screening of chemical compounds against the allosteric binding sites to discover receptor-selective drugs.

137-Plat

Novel Aspects of the Reversibility of the Antagonism at the Dopamine D2 Receptor by Antipsychotics

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All antipsychtics currently in clinical use are antagonists or weak partial agonists at the dopamine D2 receptor (D2R). Antipsychotic medication is associated with adverse effects such as extrapyramidal symptoms (EPS). The lower EPS liability of newer, so-called atypical antipsychotics, typified by clozapine, has been proposed to reflect their faster rates of dissociation from the D2R, as compared to older, typical antipsychotics such as haloperidol. This hypothesis has received increasing attention in recent years, and several pharmaceutical companies have endeavored to developed their own "fast off"-antipsychotics. However, previous studies have measured dissociation of radiolabeled antipsychotics or used modified G proteins to study receptor activation-induced calcium release, which confers certain limitations in terms of temporal resolution. We have examined antagonist dissociation in living cells, employing an assay based on the activation of G protein coupled potassium channels. This assay uses native G proteins and has higher temporal resolution than previous studies.

Our preliminary data suggest that there may be larger differences between different atypical antipsychotics than has previously been appreciated. Furthermore, the differences between atypical and typical drugs appear to relate mainly to the differential hydrophilicities of these drugs.

138-Plat

Supramolecular Architecture of Rhodopsin in Native Photoreceptors Revealed by Cryo-Electron Tomography

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Vision begins with the absorption of photons by rhodopsin, the visual pigment in photoreceptors. Rhodopsin belongs to the family of G protein-coupled receptors (GPCRs). Some family members form functional dimers or oligomers. Whether rhodopsin forms oligomers and whether these oligomers are functionally relevant is controversial [1-3]. We study rhodopsin organization in vitreous ultrathin cryo-sections of intact, dark-adapted photoreceptors by